

Sex Differences in Blood Pressure and the Kidney Cortex Transcriptome in Nonhuman Primates

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Abstract

Hypertension is a complex disease influenced by sex, and genetic and environmental factors. Blood pressure (BP) is a continuous trait that is heritable in primates, including humans and baboons. The kidneys play a role in systemically regulating BP. Sex differences in BP onset and control with antihypertensive drug therapies have been observed in humans and rodents. Hypertension studies in nonhuman primates (NHP) to date have focused on males. *We hypothesized that there are differences in renal molecular networks associated with BP in female and male primates.* Sodium-naïve female (n=8) and male (n=9) baboons were fed a low-sodium chow diet prior to and during the study. Implantable telemetry devices continuously monitored heart rate and blood pressure over 24-hours, and ultrasound-guided kidney biopsies were collected for RNA-Seq. Serum 17 beta-estradiol concentration correlated BP in females. BP in males correlated with Na⁺ intake, blood urea nitrogen, and glucose. Cell type composition of renal biopsies was consistent between females and males. Sex differences were observed in the kidney transcriptomes by principal components analysis and weighted gene co-expression network analysis. Network analysis revealed *HNF4A*, *ESR1*, *ESR2*, *SMARCA4*, *TP53*, and *NR3C1* as BP regulators in males. Our results demonstrate sex differences in primate kidney molecular networks and provide evidence of a novel link between renal transcription factors and BP regulation in males. Understanding sex differences and transcriptome variation in primate kidneys correlated with BP and clinical measures associated with BP will inform better therapies towards the goal of precision medicine for women and men.

Introduction

Cardiovascular disease is the leading cause of mortality worldwide. A major risk factor for cardiovascular disease is hypertension (HT), a complex and prevalent disease that affects nearly half of American adults(1). Early detection and diagnosis of HT can be difficult because individuals usually do not display symptoms, and while some cases of HT tied to a specific genetic variant, for the vast majority of cases, there are no clinical measures that predict when an individual will develop HT prior to an observed increase in blood pressure (BP)(1,2). This gap results in patient BP control after HT diagnosis rather than prevention of BP increase and HT(1,3). If left untreated, HT can lead to secondary complications such as prolonged damage to the circulatory and organ systems(3,4). While HT may be the result of single gene deleterious mutations, the majority of the population suffers from a more genetically complex form of the disease with variation in multiple interacting genes, which are influenced by environmental factors.

Both women and men develop HT, and while there is some overlap, sex differences have been frequently observed(1,5–13). For example, sex differences in the Renin Angiotensin Aldosterone System, specifically in androgen and estrogen receptor expression, have been observed in animals and humans(6,8–11,13). Several studies have identified sex-specific single nucleotide polymorphisms associated with HT and disparities between women and men in pharmacogenetic drug responses to available therapies(7,14–16). Additionally, women generally have lower BP than men prior to the onset of menopause (16). Despite this evidence, current HT guidelines do not include recommendations for sex-specific treatment

strategies(1). This is partially due to many studies related to HT being performed using only male subjects or grouping both sexes together for simplicity or technical limitations(17).

Baboons (*Papio hamadryas*) are genetically and physiologically similar to humans and have been used to study complex heritable human diseases, including HT and sodium-sensitive HT(18–25). Pedigreed, phenotyped and genotyped baboons at the Southwest National Primate Research Center (SNPRC) are a resource for identifying the genetic factors that regulate BP. These baboons are sodium-naïve, unlike the human population, a characteristic essential for understanding BP regulation and initiation of HT(18,21,22,24,26–28). The similarity of baboons to humans, ability to control environmental exposures, and ability to compare healthy and diseased animals enables investigation of more complex diseases that would not be easily studied in a controlled manner in human populations. These characteristics also allow for direct translation of findings to humans. In addition, recent advances of cell type-specific gene expression in the kidney allow for greater resolution of bulk RNA-Seq comparative studies to explore the underlying genetics of BP regulation(2,29).

We hypothesize there are differences in BP and renal molecular networks in female and male primates. To better understand sex differences, we selected sodium-naïve female and male baboons on a low-sodium chow diet. BP was measured continuously for 24-hours by implantable telemetry device, and transcriptome profiling of kidney cortex biopsies was performed on each animal. Sex differences were observed in clinical measures and the kidney cortex transcriptome. Unbiased analysis by Weighted Gene Co-expression Network Analysis (WGCNA) allowed us to leverage genetic variability in our cohort to identify genes associated with BP variation in males, but not females. We identified *HNF4A*, *ESR1*, *ESR2*, *SMARCA4*, *TP53*, and *NR3C1* as regulators of BP in males. The high degree of similarity between humans and baboons allows these findings to inform sex differences in BP regulation in humans. There are clear sex differences in a number of BP-related clinical measures as well as the kidney transcriptome, which are important for development of diagnostics and therapies that address this variation in women and men.

Methods

Ethics, study design, and data collection

We utilized a cohort of olive baboons (*Papio hamadryas*; Taxonomy ID 9557) maintained as part of the pedigreed baboon colony at SNPRC, located on the campus of Texas Biomedical Research Institute, San Antonio, Texas. All animal procedures were reviewed and approved by Texas Biomedical Research Institute's Institutional Animal Care and Use Committee (IACUC). SNPRC facilities at the Texas Biomedical Research Institute and animal use programs are accredited by Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), operate according to all National Institutes of Health (NIH) and U.S. Department of Agriculture (USDA) guidelines, and are directed by veterinarians (DVM). All animal care decisions were made by the SNPRC veterinarians. Enrichment was provided on a daily basis by the SNPRC veterinary staff and behavioral staff in accordance with AAALAC,

NIH, and USDA guidelines. Baboons were selected based on family history of HT as previously described (females: n=8, age 17.88 ± 1.35 years; males: n=9, age 8.10 ± 2.38 years). The KINSHIP program from Pedigree Database System (PEDSYS v. 2.0) was used with the Stevens-Boyce algorithm to calculate kinship coefficients to ensure similar degrees of relatedness among females and among males(30,31).

The baboons were raised and maintained on a standard monkey chow diet (high complex carbohydrates; low fat ("Monkey Diet 15%/5LEO," LabDiet, PMI Nutrition International) prior to study initiation. Animals were acclimated to cages for at least 8 weeks prior to study. Animals had free access to 500 g chow *ad libitum* in individual cages during feeding times. Food consumption, Na⁺ intake, body weight, and health status were monitored weekly for each animal throughout the study. In females, Na⁺ intake per kg animal was calculated as the sum of food Na⁺ intake divided by the animal weight. In males, Na⁺ intake per kg animal was calculated as the sum of food Na⁺ intake plus Na⁺ in saline infusions, divided by the animal weight. Graphical abstract created with BioRender.com

Telemetry implantation, and kidney biopsy and blood collection

All animals were preoperatively treated with ketorolac (5-30 mg), sedated with ketamine (10mg/kg, IM), and maintained on isoflurane (1.3-3.0%) anesthesia throughout telemeter implant, kidney biopsy, and blood collection procedures. Blood was drawn in EDTA tubes and separated into plasma and serum by centrifugation, and kidney cortex biopsies were collected via ultrasound guidance and flash frozen in liquid nitrogen. Animals were monitored during collections and throughout recovery. Postoperative care included observation for swelling at the surgical sight and monitoring until animal was conscious. Buprenorphine (0.2 mg/kg, SQ) was administered for post-operative pain relief immediately once animals were awake. Buprenorphine (0.2 mg/kg, SQ) was administered as needed 24-48 hours after surgery.

Diet, housing, and BP measurements in baboons

Females were run into individual cages for 4 hours (passing over an electronic weighing scale) for feeding; they were fed a chow diet (17.3 mmol Na⁺/500 g food consumed) *ad libitum* once a day for 6 weeks. Female baboons were housed in an outdoor social group with one vasectomized male (not on study) to provide full social and physical activity. Females were surgically implanted with a PhysioTel Digital implant model M10 (Data Science International (DSI)) under the external abdominal oblique muscle, and the catheter was implanted in the femoral artery to obtain 24-hour continuous BP, heart rate, temperature, and activity measurements in Ponemah software v 6.51 (DSI).

Male baboons were fed a chow diet (20 mmol Na⁺/500 g consumed) for 6 weeks and saline infused by intravenous catheter as described by Spradling-Reeves et al(21). Male baboons were tethered in individual cages, and intravenous catheters were surgically implanted as previously described by Carey

et al. to obtain 24-hour continuous BP, heart rate, temperature, and activity measurements in Ponemah software v 5.2 (DSI)(26).

Clinical measures related to kidney function and BP

Blood chemistry panels were performed to measure hematocrit, glucose, total protein, blood urea nitrogen (BUN), creatinine, serum Na⁺, and serum K⁺ as described (21). Sodium outtake, plasma renin activity, plasma aldosterone, and PAH clearance were measured as described (21). The cytokine and chemokines IL-1Ra, IL-2, IL-6, IL-8, IP-10, MCP-1, MIP-1a and sCD40L were measured in plasma using a custom Luminex assay as detailed by Giavedoni(32). Serum 17 beta-estradiol was measured in female baboons with the 17 beta Estradiol ELISA Kit (Abcam); human female serum (age 28 years) was used as a positive control.

Telemetry data analysis

Ponemah software was used for BP analysis with signal acquired at a logging rate of 10 seconds per minute. Data were reduced to 10-minute periods throughout the study for mean arterial BP (MAP), systolic BP, diastolic BP, and heart rate. The data were calculated for 24-hours, as well as 12-hour daytime (6:00 am-6:00 pm) and 12-hour nighttime intervals (6:00 pm-6:00 am). BP data reported here are based on the last day of recording on chow diet before kidney cortex biopsy collection.

RNA isolation, library preparation, and sequencing

RNA was isolated from kidney biopsy samples using the Direct-zol RNA Miniprep Plus Kit (Zymo Research). Tissue was homogenized in 600 µL of TRI reagent (Zymo Research) using a BeadBeater (Biospec Products) for 3 x 30 sec with RNA purification according to the Direct-zol RNA Miniprep Plus Kit instructions. RNA was quantified by Qubit RNA BR Assay Kit (Invitrogen), and RNA integrity was determined with an RNA ScreenTape Kit on the TapeStation 2200 system (Agilent). cDNA libraries were prepared using the KAPA Stranded mRNA-Seq Kit (Roche). Libraries were quantified using a KAPA Library Quantification Kit (Roche), and quality was determined with a DNA ScreenTape Kit on the TapeStation 2200 system (Agilent). Sequencing was done using a HiSeq 2500 (Illumina).

RNA-Seq data processing

Female and male transcripts were processed together in Partek[®] Flow[®] (Partek[®], Inc.) with a minimum read length of 25 and minimum Phred 30. Reads were aligned to the olive baboon genome (papAnu2.0;

March 2012).

Transcripts were annotated to identify kidney-specific cell types as shown by Park et al(2). The full transcript list generated in Partek[®] Flow[®] was imported into Partek[®] Genomics Suite[®] (Partek[®], Inc) for principal component analysis (PCA).

Weighted gene co-expression network analysis

Transcripts were filtered for expression values totaling <17 among all samples and filtered based on coefficient of variation for values equal to 0 prior to sex-specific WGCNA. The R package WGCNA was run with default settings which assign each transcript to a single module as described by Langfelder and Horvath(33).

Pathway and network analysis

Pathway and causal network enrichment analyses of genes in significant modules were performed using Ingenuity Pathway Analysis software (IPA; Ingenuity[®] Systems).

Using search terms “hypertension” and “blood pressure”, variants were downloaded from the GWAS Catalog and merged with genes in significant WGCNA modules (Galaxy Genome, <https://usegalaxy.org/>) to identify genes with variants previously associated with BP(34).

Statistics

Clinical Measures

Mean values and standard deviations for Na⁺ intake are reported as the average over 5 days prior to biopsy collection. Unpaired t-tests with or without Welch's correction were performed on each of the clinical and BP traits as appropriate. Spearman correlation was performed on each of the clinical and BP traits. Data are expressed as mean, mean ± SD, or correlation r value, and were considered to be statistically significant if p-value < 0.05.

Transcripts

TMM+1 was used for normalization of trimmed and aligned transcripts. Multiple unpaired t-tests with FDR Two-stage setup (Benjamini, Krieger, and Yekutieli) for multiple testing were performed on cell type-specific transcripts between females and males. PCA was performed on normalized transcripts with ellipsoids representative of 2 standard deviations away from the mean. WGCNA was performed using

Pearson correlation with a p-value cutoff < 0.05. Correlation directionality was calculated by subtracting expression values across transcripts of animals with the lowest BP from animals with the highest BP for transcripts correlated with BP and input into as expression values for IPA network analysis. Upstream analysis and causal network regulator lists were ranked by p-value. Data are expressed as mean, mean \pm SD, or correlation r value, and were considered to be statistically significant if p-value < 0.05.

Results

BP in female and male baboons

Pedigreed female and male baboons were identified from familial lines with variation in BP, which is potentially related to genetic variation in regulation of BP (Additional file 1 tab S1). Telemetry measurements of MAP, systolic BP, and diastolic BP showed that these are continuous traits among this population of pedigreed baboons for both sexes over 24-hours and day/night intervals. This pattern is represented by the variation exhibited in 24-hour MAP (Figure 1). Sex differences were significant in daytime MAP, daytime systolic BP, 24-hour systolic BP, and nighttime systolic BP between females and males (Additional file 1 tab S2).

Clinical measures in female and male baboons

Significant differences between female and male baboons were observed in serum Na⁺ (p-value=0.012), BUN (p-value<0.001), and glucose (p-value=0.0018) (Additional file 1 tab S2). BP was significantly different between female and male baboons for 24-hour systolic BP (p-value<0.0001), daytime MAP (p-value=0.0107), daytime systolic BP (p-value<0.0001), and nighttime systolic BP (p-value=0.0001) (Additional file 1 tab S2). Serum 17 beta-estradiol concentrations were significantly correlated with 24-hour MAP (p-value=0.0458), nighttime MAP (p-value=0.0458), 24-hour systolic BP (p-value=0.0279), and nighttime systolic BP (p-value=0.0279) in females; but there was no significant correlation between BP and age, Na⁺ intake; blood measures of BUN, total protein, glucose, hematocrit; or serum measures of K⁺, Na⁺ (Table 2, Additional file 1 tab S3). Males showed negative correlation between 24-hour diastolic BP, daytime diastolic BP, and nighttime diastolic BP with Na⁺ intake (p-values =0.0143, 0.0112, 0.0239). Plasma IP-10 was positively correlated with BUN (p-values =0.047); and a positive correlation between nighttime systolic BP and glucose (p-value=0.0313) was observed (Table 2, Additional file 1 tab S3). Males also demonstrated 24-hour MAP and nighttime MAP, negatively correlated with PAH clearance (p-value=0.014, 0.021). 24-hour MAP, 24-hour diastolic BP, daytime diastolic BP, and nighttime diastolic BP negatively correlated with plasma IL-8. Plasma IL-8 was also negatively correlated with plasma IL-6 (p-value=0.006).

Cell type-specific gene expression in kidney cortex biopsies

Transcript quality was similar between female and male samples, but females had a lower average transcript alignment and greater average read lengths than males (Additional file 1 tab S4). All samples met quality standards (total reads, alignment, coverage, read length, depth, read quality) to proceed with downstream analysis (Additional file 1 tab S4). Kidney cortex RNA-Seq data showed representation of all kidney cell types previously characterized by Park et al (data not shown)(2). Thick ascending limb and proximal tubules had the greatest expression among all samples, suggesting these may be the predominant cell types within the kidney cortex (Additional file 1 tab S6). Abundance of kidney-specific genes (n=40) did not differ significantly between females and males (Figure 2A, Additional file 1 tab S5), indicating consistency of biopsy collection among study animals.

Sex differences in kidney cortex transcriptome

Although kidney cortex-specific genes did not differ between females and males, PCA of all transcripts for females and males revealed the top principal components of 24%, 11.6%, and 9.03%, which account for 44.6% variation in the samples and demonstrate two clusters with sex-specific profiles (Figure 2B). Coefficient of variation for transcripts in females and males demonstrated sex differences in variation in gene expression and also revealed sex differences in distribution of variation in the transcriptome (Figure 2C, Additional file 1 tab S6).

Relation of kidney transcriptome to BP-related clinical measures

Each WGCNA module contained transcripts co-correlated with each other, the resulting modules were then tested for correlation with each quantitative trait, e.g., daytime systolic BP, blood glucose, etc. (Figure 3, Additional file 2, Additional file 1 tab S7, S8). No modules were significantly correlated with BP traits in females (Additional file 2, Figure 1). Analysis of males revealed 3 modules (greenyellow, n=750 transcripts; yellow, n=1311 transcripts; magenta, n=915 transcripts) were positively correlated with BP traits: nighttime MAP, 24-hour systolic BP, nighttime systolic BP, 24-hour diastolic BP, nighttime diastolic BP (p-values = 0.02, 0.03, 0.03; 0.05, 0.01, 0.04, respectively) (Figure 3). These modules contained 42 renal-specific genes and 237 genes with GWAS variants associated with BP (Additional file 1 tab S8).

Gene networks in male kidney cortex biopsies

In males, upstream regulators and master regulators *HNF4A*, *ESR1*, *ESR2*, *SMARCA4*, *TP53*, *NR3C1* were the most significant with the greatest number of downstream targets for genes in modules correlated with BP (pink, greenyellow, magenta, and yellow) (Figure 4, Additional file 1 tab S9, S10). Network analysis of *HNF4A*, *ESR1*, *ESR2*, *SMARCA4*, *TP53*, and *NR3C1* demonstrated they had overlapping direct connections forming a single gene network (Figure 3). Proximal tubule cells were

identified as an important cell type in these networks, with the cell type-specific expression of *HNF4A*. Predicted upstream regulators included 4 kidney cell types (proximal (general)-*HNF4A*, inner medulla-*ELF5*, podocyte-*WT1*, and plasma cell-*IRF4*) in the network and included *ESR1* among 14 genes with GWAS associations as upstream regulators in modules correlated with BP in males (Figure 4, Additional file 1 tab S9). Furthermore, targets of these upstream activators and inhibitors included cell membrane-localized potassium and calcium ion channels, as well as targets related to proliferation and tissue remodeling (Additional file 1 tab S9, S10).

Discussion

HT impacts approximately 50% of the US adult population(1). Currently, there are no sex-specific diagnostics or treatment guidelines, despite numerous studies in humans and rodents showing sex differences in previously identified regulators of HT. It is well-known that HT is a complex disease with many players in many tissues contributing to the overall molecular mechanisms underlying BP regulation. The baboon has been used as a model for HT due to the genetic and physiological similarity to humans(18–25). Previous studies have focused on male baboons; therefore, sex differences in kidney gene networks underlying BP regulation have not been assessed in primates, including humans and baboons(18–25). In this study, we investigated sex differences in kidney gene networks associated with BP and BP-related clinical measures in female and male baboons to nominate regulatory networks central to BP regulation. Establishing whether there are sex differences in kidney gene networks associated with BP and BP-related traits is essential for developing more effective diagnostics and therapies for both women and men.

A major finding of this study was that BP differences between females and males varied by measurement type and time of day. Sex differences were observed in 24-hour systolic BP, daytime MAP, nighttime diastolic BP, and nighttime systolic BP, with females having lower BP than males. Females demonstrated correlation between serum 17 beta-estradiol concentration and 24-hour MAP, nighttime MAP, 24-hour systolic BP, and nighttime systolic BP but no other clinical measures. The role of 17 beta-estradiol in female primate BP regulation still needs further investigation. BP correlated with a number of clinical measures in males: 24-hour diastolic BP, daytime diastolic BP, and nighttime diastolic BP with Na⁺ intake; 24-hour MAP, daytime MAP, and 24-hour systolic BP with BUN; as well as nighttime systolic BP with glucose. We also observed 24-hour MAP and nighttime MAP, negatively correlated with PAH clearance. 24-hour MAP, 24-hour diastolic BP, daytime diastolic BP, and nighttime diastolic BP negatively correlated with plasma IL-8. Daytime diastolic BP was positively correlated with plasma IL-6. Interestingly, plasma renin activity and plasma aldosterone did not correlate with any BP measures. This provides evidence that these typical BP-related clinical measures are not as informative for females as for males, and emphasizes the importance of sex-specific markers of HT pathology and treatment plans.

We demonstrated consistency in kidney biopsies by cell composition, diet, as well as both time of day and duration of collection of telemetry data between females and males - all indicating that differences in the study design did not account for kidney transcriptome differences. In addition, we showed sex

differences in the primate kidney transcriptome(2,35–37). WGCNA of kidney cortex RNA-Seq data was used as an unbiased approach to identify sex-specific transcripts correlated with BP. This analysis is the most appropriate considering the clear variability observed in the BP and transcriptome data, and adds statistical power that would be lost by binning for pairwise comparison. Our analysis did not reveal any WGCNA transcript modules correlated with BP in females, demonstrating there may be other important players for female BP regulation not found in kidneys with a low sodium dietary environment. WGCNA of males revealed 4 transcript modules strongly correlated with BP, further supporting the observed sex differences in gene expression.

Unlike the females, identification of significant WGCNA modules in males correlated with BP allowed us to move forward with the dataset and perform network analysis. In males, gene modules positively correlated with BP contained overlapping networks with predicted regulation by *ESR1*, *HNF4A*, and *TP53* which have been previously identified as important players related to BP regulation in the kidney (38,39). While some of these players have been associated with the RAAS, neither BP or the kidney cortex transcriptome correlated with plasma renin or aldosterone levels (38,39). Indicating BP regulation in the kidney is not entirely driven by the RAAS.

However, expression of *ESR1*, *HNF4A*, and *TP53* did not directly correlate with BP. Activity of these gene products is known to be dependent on post-translational modifications, i.e. gene expression does not directly inform their protein activity. These findings indicate the need to verify the roles of these master regulators for BP regulation with assessment of post-translational modifications and their impact on these regulatory networks(41–44).

Network analysis of renal transcripts in male modules correlated with BP identified several genes and upstream regulators related to maintenance of ion channels (e.g., *CLCN3*, *KCNK3*, *CLIC3*, *KCNMA1*), insulin regulation (*TCF7L2*, inhibited), and transcription factor HNF4A (predicted activated) which is exclusively expressed in the proximal tubules and involved in regulating transport proteins(45–54). We also found estrogen and glucocorticoid receptors (*ESR1*, predicted activated; *ESR2*, predicted inhibited; *NR3C1*, inhibited) as upstream regulators of genes in modules correlated with BP, which influence the reabsorption of calcium, potassium, sodium, and glucose in the kidney(37,46–48,53,55). Inclusion of these genes in BP correlated modules connects hormone receptors, sodium management and glucose homeostasis with BP regulation at the molecular level in males. *TCF7L2* and *ESR1* in our results were previously shown by GWAS to have variants associated with BP. Due to sample limitations, we were unable to further investigate the role of estrogens in male BP regulation. Lastly, we identified upstream regulators of transcripts related to cell proliferation and chromatin remodeling (*TP53*, predicted activated; *SMARCA4*, inhibited; *NOTCH1*) in modules correlated with BP(56–58). The cross talk between ion and glucose homeostasis suggests male primates with higher BP in a low sodium dietary environment have a lower capability to remodel their vasculature, possibly leading to less flexibility and increased arterial stiffness in response to damage and stress compared to animals with lower BP.

This is the first study to characterize the kidney cortex transcriptome of adult female baboons, allowing comparison with males, and the first to nominate renal networks that influence BP regulation in females. Furthermore, this work provides evidence for the importance of including female subjects in research to understand differences in BP regulation. Follow-up studies to validate mechanisms of regulatory networks are needed for translation of findings to understanding regulation of BP in women.

Study Limitations: For this comparison, there were a few differences in the study design for females versus males. Males were housed in individual cages and females were housed in a social group. Housing differences were due to limitations of telemetry transmitter distances when the study of male baboons was conducted; whereas, newer telemetry technology used for the females allowed collection of data from animals in social groups. In both studies, animals were acclimated to housing and social groups (males had visual contact with all other males in the study) for a minimum of 2 weeks with BP monitored for stability prior to study initiation. Although study design differed slightly with male sodium intake via saline solution, no studies to date indicate route of administration of sodium impacts BP or other BP regulating players. The females in this study were premenopausal based on age, menstrual cycles, and 17 beta-estradiol levels. If age were a factor influencing BP, we would expect females to have higher BP than males, as seen when comparing older versus younger humans, which is contrary to our study results. While we cannot entirely eliminate the possibility that age differences influenced our results, we show that different mechanisms underly renal BP regulation in older adult females compared with middle aged adult males. Even with these limitations, our findings support the need to further understand sex-differences in renal molecular mechanisms influencing BP.

Declarations

Ethics approval and consent to participate

Details on ethics for animal use in this study are detailed in the section “ethics, study design, and data collection”

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the locations detailed below:

RNA-Seq dataset: NCBI GEO GSE166295.

GWAS data were derived from: GWAS Catalog; <https://www.ebi.ac.uk/gwas/> (34)

Cell type specific gene identifiers were derived from Park et al(2).

Competing interests

None

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Tables

Clinical measures in female and male baboons

Trait (Units of measure)	Female Average	Female SD	Female Min	Female Max	Male Average	Male SD	Male Min	Male Max	P value	95% confidence interval
Age (years)	17.9	1.4	15	19	8.2	2.5	6	13	<0.0001	-11.73 to -7.576
Sodium intake (mmol Na ⁺ /animal weight kg)	0.69	0.24	0.51	1.22	0.88	0.08	0.78	1.01	0.0587	-0.008876 to 0.3939
Serum K ⁺ (mmol/L)	3.54	0.53	2.8	4.7	3.9	0.35	3.35	4.5	0.1237	-0.1165 to 0.8526
Serum Na ⁺ (mmol/L)	139.38	2.83	135	144	142.94	2.32	139.5	147	0.012	0.9074 to 6.232
Creatinine (mg/dL)	1.03	0.22	0.8	1.4	1.18	0.13	1.05	1.4	0.0978	-0.03163 to 0.3372
BUN (mg/dL)	10	2.2	7	14	17.78	1.44	16	20.5	<0.0001	5.781 to 9.775
Total protein (g/dL)	6.93	0.54	6.2	7.7	7.19	0.2	6.85	7.45	0.1848	-0.1437 to 0.6826
Glucose (mg/dL)	125.63	28.9	89	169	83.94	15.3	59.5	101.5	0.0018	-65.18 to -18.18
Hematocrit (%)	38.7	5	29.8	48.1	41.9	3.1	37.5	47.4	0.1295	-1.051 to 7.446
24-hour diastolic BP (mmHg)	71.9	7	60.9	84.1	70.6	4.2	62.9	76.2	0.6276	-7.271 to 4.529
24-hour MAP (mmHg)	89.5	8.1	77.2	100.9	94	4.1	86.7	100.8	0.1604	-1.992 to 10.98
24-hour systolic BP (mmHg)	111.5	8.7	99.2	123	136.4	5.3	132.3	145.9	<0.0001	17.18 to 32.74
Daytime diastolic BP (mmHg)	71.9	7.2	61.9	83.9	74	4.5	63.7	78.9	0.4937	-4.369 to 8.536
Daytime MAP (mmHg)	89.5	8	78.5	100.3	98.7	5	88	106.1	0.0107	2.485 to 16.06

Daytime systolic BP (mmHg)	111.7	8.5	101	122.4	142.5	6	135	150.8	<0.0001	23.25 to 38.27
Nighttime diastolic BP (mmHg)	71.9	7.2	60.2	84.2	68.3	4.5	62	75.9	0.2193	-9.818 to 2.443
Nighttime MAP (mmHg)	89.5	8.4	76.3	101.3	90.8	4.2	85.3	97.5	0.6823	-5.430 to 8.075
Nighttime systolic BP (mmHg)	111.3	9.2	98.1	123.5	132.4	5.9	126.6	142.8	0.0001	12.76 to 29.34

Table 1. Clinical measures in female and male baboons. Unpaired two-tailed t-tests or unpaired two-tailed t-tests with Welch's correction were performed on each clinical trait in female and male baboons. P values represent the difference between sexes and were considered significant if less than or equal to 0.05.

Correlations between clinical measures in female and male baboons

Correlation variables		Female		Male	
		r	p-value	r	p-value
17 beta-estradiol	Serum K ⁺	0.86	0.009	-	-
17 beta-estradiol	24-Hour MAP	0.74	0.046	-	-
17 beta-estradiol	Nighttime MAP	0.74	0.046	-	-
17 beta-estradiol	24-Hour systolic BP	0.79	0.028	-	-
17 beta-estradiol	Nighttime systolic BP	0.79	0.028	-	-
Na ⁺ intake	Serum Na ⁺	-0.67	0.076	0.88	0.003
Na ⁺ intake	24Hour diastolic BP	-0.06	0.897	-0.79	0.014
Na ⁺ intake	Daytime diastolic BP	-0.04	0.943	-0.81	0.011
Na ⁺ intake	Nighttime diastolic BP	-0.06	0.897	-0.75	0.024
Creatinine	Age	0.05	0.909	0.87	0.003
BUN	Serum K ⁺	0.02	0.960	-0.77	0.018
BUN	24-Hour MAP	-0.10	0.830	-0.70	0.043
BUN	Daytime MAP	-0.12	0.793	-0.76	0.023
BUN	24-Hour systolic BP	0.00	1.000	-0.76	0.023
Glucose	Nighttime systolic BP	-0.26	0.536	0.73	0.031

Table 2. Correlations between clinical measures in female and male baboons. Correlation variables are listed between clinical measures for each comparison. Spearman r values and p-values are presented for female (N=8) and male baboons (N=9).

Figures

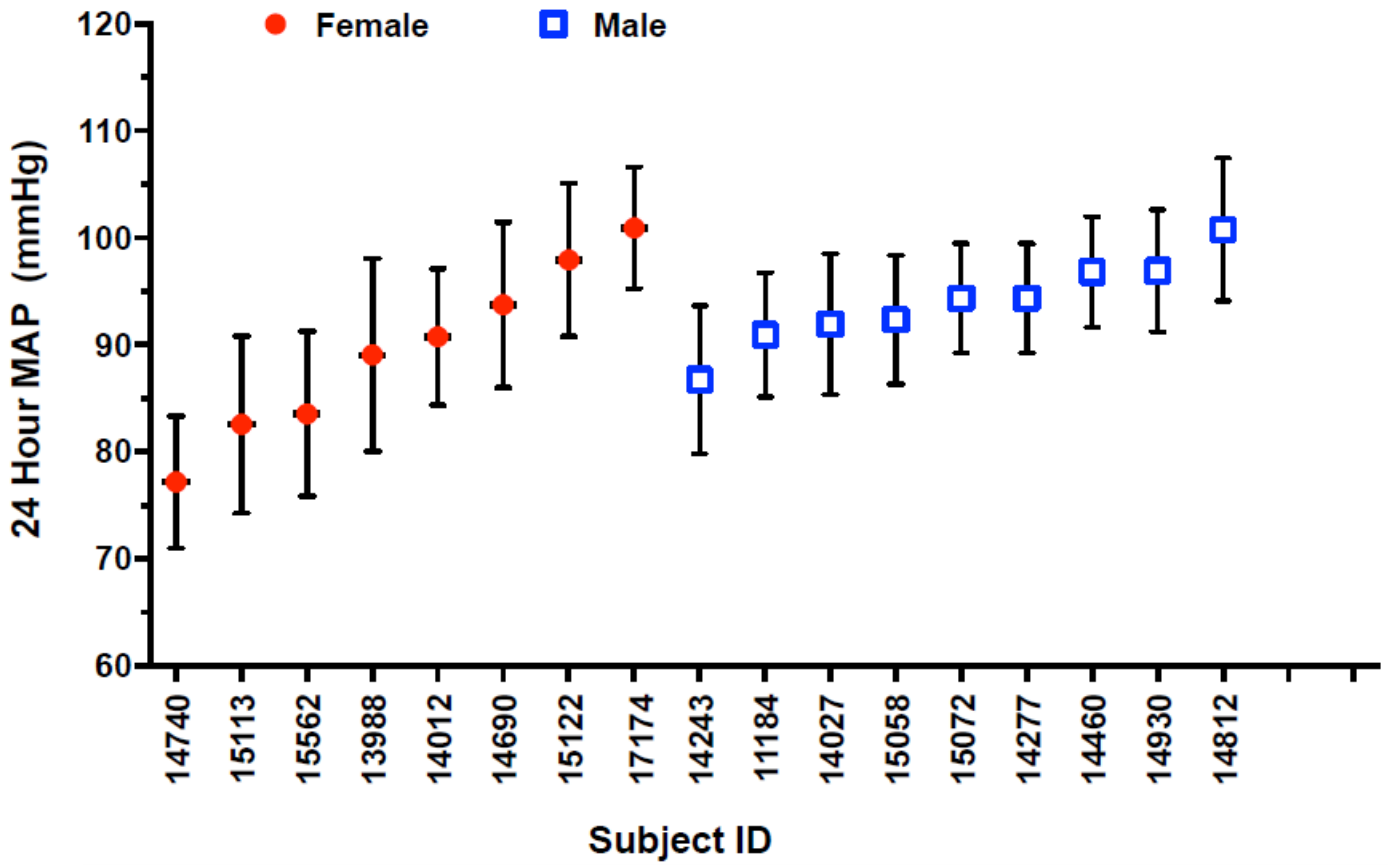


Figure 1

Mean arterial pressure (MAP) over 24-hours in female and male baboons. Animal ID is represented for each animal along the x-axis, and 24-hour MAP (mmHg) is shown along the y-axis. Mean values for each animal are represented as columns, and bars represent standard deviation.

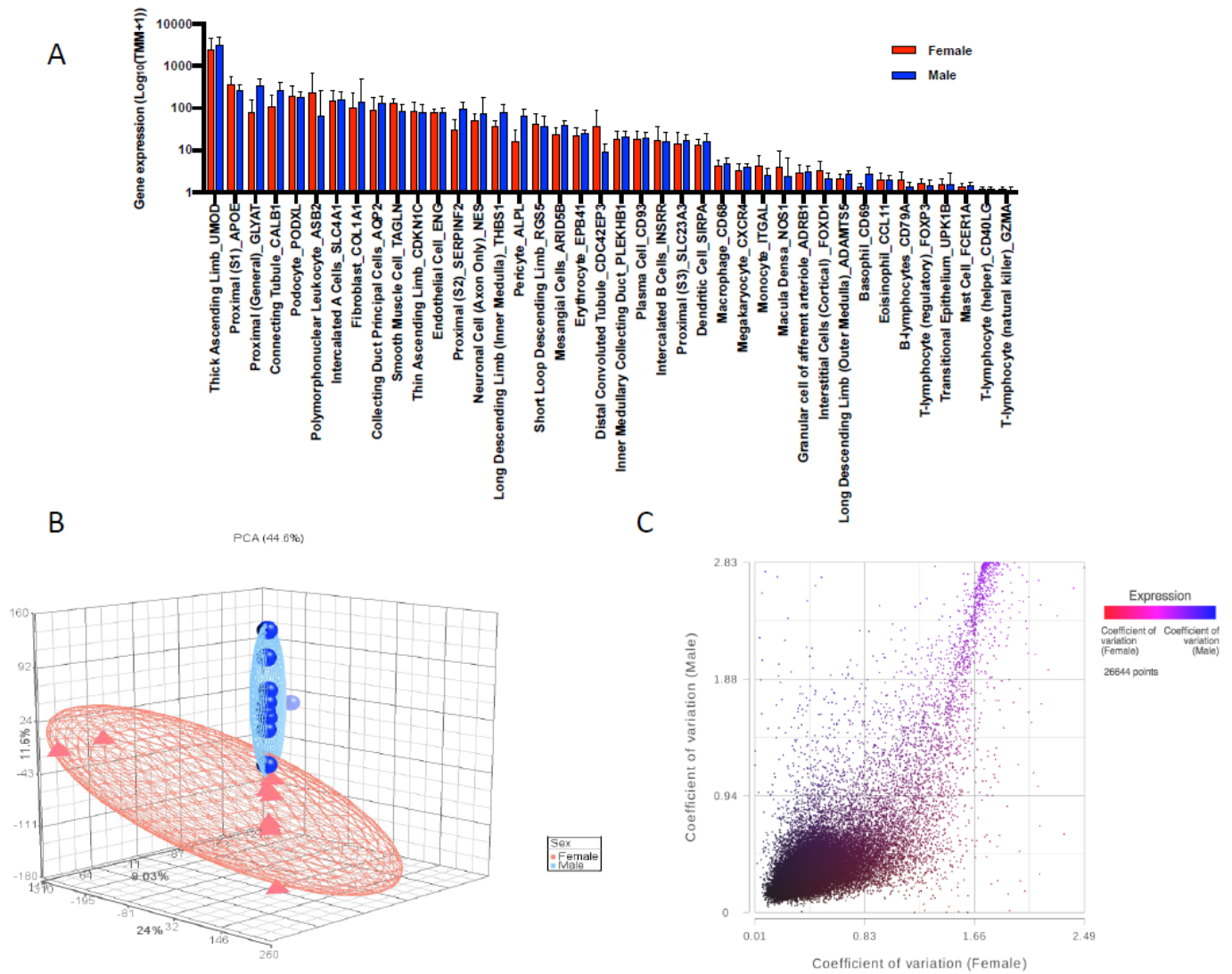


Figure 2

Variation in gene expression of female (n=8) and male (n=9) kidney cortex biopsies. A. Cell type distribution by gene expression in female and male (n=9) kidney cortex biopsies. Mean expression values reported for each gene as the log(TMM+1) are represented as columns, and bars represent standard deviation. The x-axis is labeled according to cell type and corresponding gene name. B. Principal components analysis of female and male kidney cortex transcriptomes. Top principal components total 44.6% represented with each axis as follows: 1=24%, 2=11.6%, 3=9.03%. Each female transcriptome is represented as a red triangle and each male transcriptome as a blue sphere. Ellipsoids denote 2 standard deviations from the mean for each group. C. Coefficient of variation in 26,644 female and male kidney cortex biopsy transcripts. Each point demonstrates a single transcript with a particular coefficient of variation value in females on the x axis (n=8) and males (n=9) on the y axis. The color of each point indicates if the transcript expression level is greater in females (red) or males (blue).

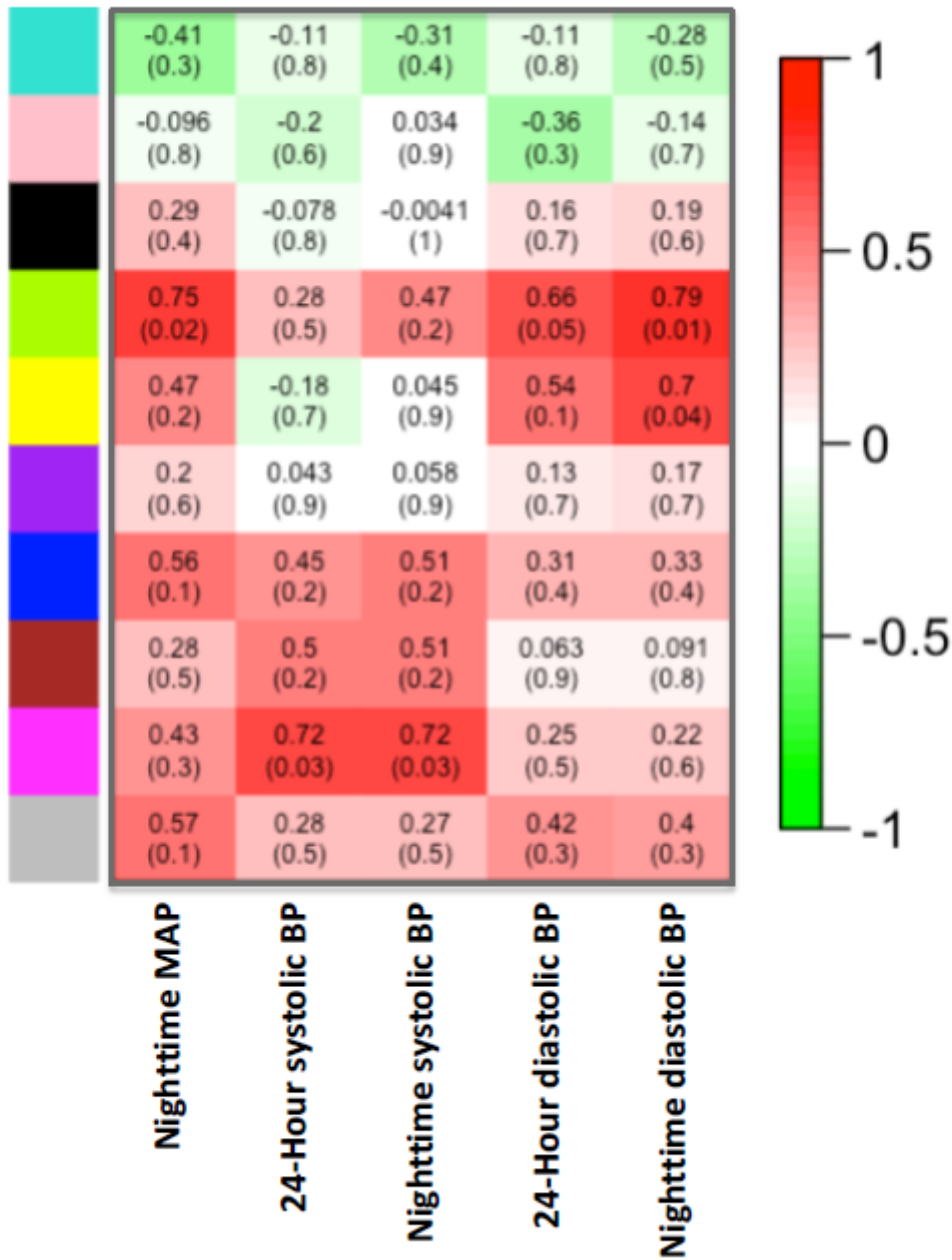


Figure 3

WGCNA of traits correlated with transcript modules in male baboons on low-sodium chow diet (n=9). Each colored block along the left side represents a module of transcripts correlated with each other. Top-values in each block represent correlation, bottom values in each block represent p-values for each transcript module and trait. Color scale on the right side of the heatmap corresponds to correlation r values of each square. Abbreviated measures along the x-axis represent the following measures: nighttime MAP (mmHg), 24-hour systolic BP (mmHg), nighttime systolic BP (mmHg), 24-hour diastolic BP (mmHg).

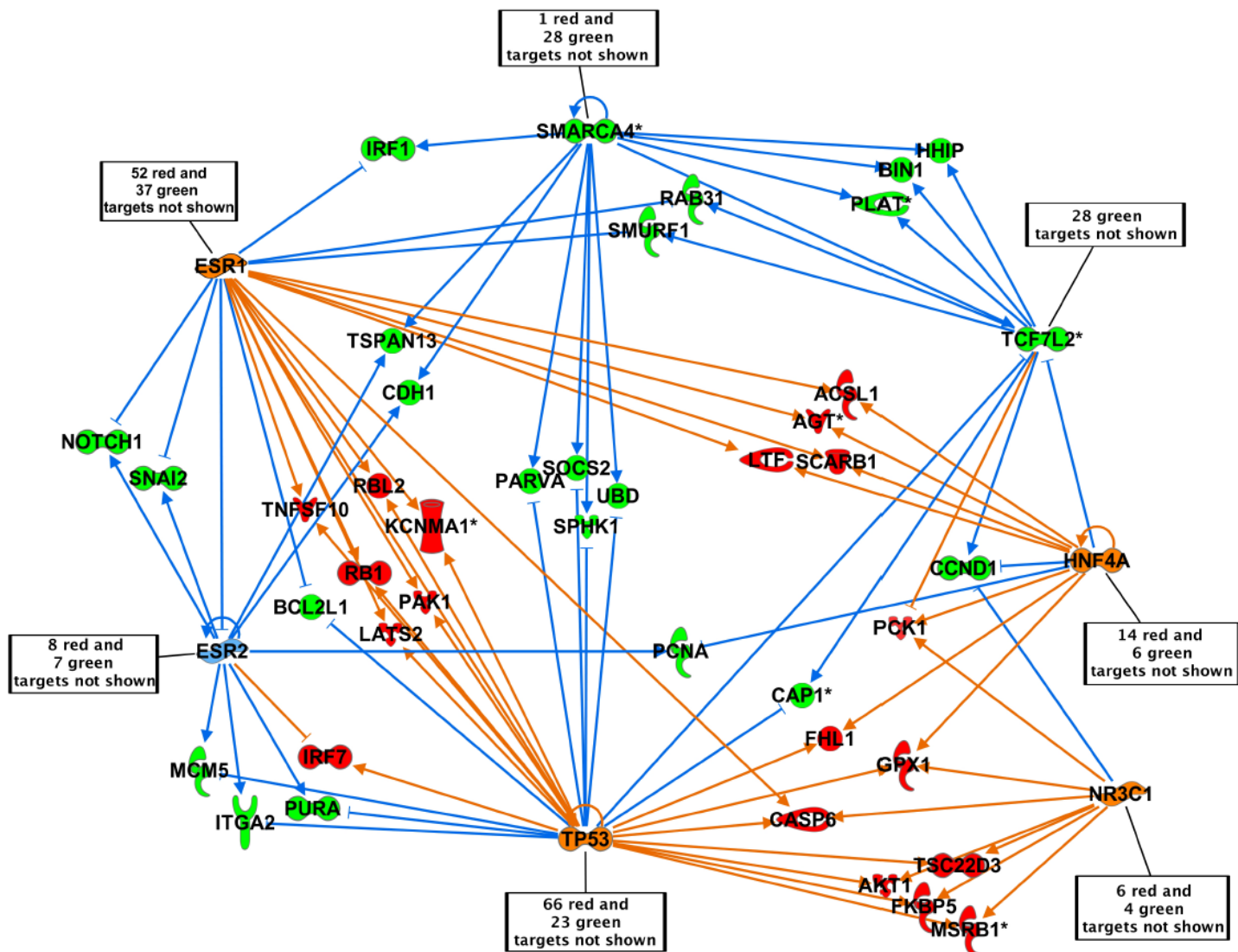


Figure 4

Network analysis of WGCNA transcripts correlated with BP in males. Upstream regulators of transcripts and targets are shown; upstream regulators are identified as having a box indicating “targets not shown” branching off. Molecules in orange are genes predicted as activators, and genes in red are positively correlated with BP in the dataset. Molecules in blue are genes predicted as inhibitors, and genes in green are negatively correlated with BP in the dataset. Arrows indicate direction of activation of downstream gene, and T lines represent inhibition of downstream gene. Orange lines indicate target activation supported by the literature, blue lines indicate target inhibition supported by the literature.

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